A BIOLOGICAL EXAMINATION OF DISTILLED WATER.

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The accuracy of results obtained in many of the present lines of physiological research depends so entirely on the purity of the chemicals used, including water, that I may be pardoned for publishing an account of work which otherwise would have little significance, the toxicity of metal-distilled water having been repeatedly shown. The results obtained are, however, applicable in a practical way at Woods Hole, where it is difficult to get good distilled water.

Two years ago I arranged an automatic still in which the water was boiled in a block tin vessel but the dry steam received and condensed in glass. It was thought that if the water did not touch metal after condensation, contamination by ions would be avoided. The distillate proved, however, to be decidedly toxic. I could not believe that dry steam carried with it an appreciable quantity of metal and, therefore, decided to find out where the trouble lay.

In these experiments, sea-water was condensed to a known fraction of its volume and then measured portions brought back to the original volume by adding the distilled waters to be tested. Tap-water was also used for comparison; sometimes, without treatment; sometimes, sterilized without boiling; in some experiments a portion was boiled away and the remaining portion used.

To the artificial sea-waters prepared as above equal amounts of fertilized *Arbacia* eggs were added and the development observed. The percentage of plutei developed in the different solutions as compared with natural sea-water was usually taken as a measure of purity. In other cases, the percentage of blastulæ was the standard; or the percentage of segmentation in a given time; or the length of time that any larvæ remained alive. The records of some typical experiments follow:

Experiment 6.—250 c.c. sea-water were concentrated to 75 c.c. To each 15 c.c. of this solution 35 c.c. of the waters named

in column 2 were added. To the dishes were added equal amounts of *Arbacia* eggs in 16–32-cell stage.

No.	Description of Water.	20 Hours.	28 Hours.	54 Hours.
1	Double distilled in glass.	Early gastrulæ. Behind control.	Behind control.	Plutei.
2	Tap.	Blastulæ.	Many going to pieces.	All dead.
3	Tap boiled down one half.	Slightly behind No. 1. But much better than No. 2.	Almost as good as No. 1.	Plutei as well developed as No. 1; not so numerous.
4	Control in sea water.			Advanced plutei.

Experiment 11.—Two liters of sea-water were evaporated to 250 c.c. To 10 c.c. portions of the resulting solution were added respectively 70 c.c. of waters described below. Equal quantities of *Arbacia* eggs were added. Three days later the percentages of plutei were ascertained by first killing the cultures and then making counts in a watch glass marked off into squares.

	Per cent. Plutei.
1. Control, 80 c.c. sea-water.	90
2. Artificial sea-water made from tap-water.	0
3. " " " " " tap-water heated to 60° in closed flask.	0
4. " " " " " distilled water from Metcalf's, Boston.	16[1]
5. " " " " " water boiled in copper without H_2SO_4 but condensed in glass.	32[1]
6. Artificial sea-water made from water boiled in copper with H_2SO_4 but condensed in glass.	78[2]
7. Artificial sea-water made from water distilled wholly in glass; first part thrown away.	66
8. Artificial sea-water made from water distilled in patent automatic metal still.	5[3]
9. Artificial sea-water made from water distilled wholly in glass, with $K_2Cr_2O_7$ and H_2SO_4.	87
10. Artificial sea-water made from water double distilled in glass, first distillates rejected.	90
11. Artificial sea-water made from "Pureoxia" distilled water.	3[4]

Experiment 8.—Two liters of sea-water were boiled down to 400 c.c. Ten c.c. of this concentrated sea-water were added to 40 c.c. of each of the waters listed in column 2. To every dish was added an equal amount of fertilized *Arbacia* eggs on July 10, 1903.

[1] Nearly all small.

[2] Some small; others as good as control.

[3] All very small and imperfect.

[4] All very small and imperfect. Only experiment with this brand.

EXPERIMENT 8.

No.	Description of Water.	Condition of Cultures on Dates Named.					
		July 11.	July 12.	July 16.	July 20.	July 23.	July 28.
1	Tap.	No larvæ.					
2	1st 50 c.c. distilled in glass from 1,000 c.c. tap.	Many gastrulæ, but not so good as No. 6.	Many plutei; not so good as No. 3.	Some alive; not so good as No. 4.	All dead.		
3	2d 50 c.c. ditto.	Ditto.	Not so good as control. 40 per cent. plutei.	Many alive.	Some alive.	All dead.	
4	3d 50 c.c. ditto.	Better than No. 2.	As good as control.		Many alive.	Many alive.	All dead
5	Distilled in glass from tap water, which had been boiled in open vessel.	Fine lot of gastrulæ.	As good as control.	Many alive and lively.	All dead.[1]		
6	1st 50 c.c. distilled from 1,000 c.c. tap + 5 grms. potassium bichromate and 10 c.c. H_2SO_4.	Ditto.	Better than control.	Much better than No. 3 or No. 9.	Many alive; not so good as No. 8.	Many alive.	All dead.
7	2d 50 c.c. ditto.	Ditto.	Ditto.	Ditto.	Ditto.	Ditto.	All dead.
8	Double distilled in glass, first distillate in each case thrown away.	Ditto.	As good as control or better.		Fine lot alive.	Fine lot alive.	All dead.
9	Distilled water from Metcalf, Boston.	Ditto.	About like No. 2.	Many alive.	All dead.		
10	Same as No. 5 + 1 c.c. $\frac{m}{10}$ NH_4OH	No development.					
11	Control, in 50 c.c. sea-water.	Fine lot of gastrulæ.	50 per cent. are good plutei.	Some alive.	All dead.[1]		

[1] Not usual in water prepared in this way. Should have lived longer.

From the above and other experiments I can make the statements enumerated below, which apply, of course, to *Arbacia* only but point to the necessity of caution in using distilled water on other organisms. It is true also that the results are most applicable to Woods Hole conditions and to the tap-water used as a basis of the distilled water there. It is probable that the toxicity is due to ammonia although this was not proven. It is certain from experiments made that *Arbacia* larvæ are very sensitive to that substance.

1. Tap-water is decidedly, although variably, toxic. The toxicity is not lost by sterilization but is greatly reduced by boiling the water for a long time, say until one third has been boiled away. The residue in such cases is less toxic than some distilled waters, particularly commercial brands and that from automatic stills.

2. Water from ordinary automatic stills, whether metal or entirely glass, is toxic.

3. The commercial distilled waters used by me were toxic, often in high degree.

4. In distilling water in the ordinary way from glass, the first one tenth distilled over is decidedly toxic, the second tenth less so, the third tenth still less so. The fourth tenth is of good quality.

5. The best distilled water used was produced by double distilling in glass, the first fourth distilled over in each distillation being rejected.

6. Nearly as good water was produced by single distillation from tap-water to which H_2SO_4 and $K_2Cr_2O_7$ had been added.

7. If a little H_2SO_4 is added to the tap-water to start with, an excellent quality of distilled water may be produced from an automatic still consisting of a copper vessel and glass condenser, the arrangement being such that none of the condensed water touches the metal. This water is practically free from ions or toxic volatile substances. It is much better than water double distilled in glass in the ordinary way, unless in the later case a large proportion of the product be thrown away. Such an automatic still is recommended for use at Woods Hole.

8. It was noted in a number of cases that *Arbacia* lived longer

in an artificial sea-water prepared from a good quality of distilled water than in natural sea-water. It is probable that the volatile toxic substance (ammonia?) exists in sufficient quantity in sea-water to have an appreciable effect.

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